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### Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: OLIGONUCLEOTIDE PRIMERS THAT DESTABILIZE NON-SPECIFIC DUPLEX FORMATION AND USES THEREOF

### (57) Abstract

The present invention relates to the demonstration that a modification of a homopolymeric stretch in an oligonucleotide, or primer, improves the discrimination for binding of such a modified oligonucleotide or primer to its complementary homopolymeric target sequence, as compared to a non-homopolymeric sequence. More specifically, an oligo d(T) primer having two of the thymine bases substituted by 3-nitropyrrole were used in a poly A primed cDNA synthesis experiment to demonstrate an improvement in discrimination between the priming of cDNA synthesis from bona fide poly A sequence as compared to internal A-rich sequences. The present invention relates to modifications of homopolymeric sequences in oligos, decreasing the ridging bonding capacity, in general, since other modifications, such as an oligo d(T) primer substituted with 2' deoxyinosine was also shown to improve the discrimination between the binding to a bona fide poly A tail as compared to A-rich sequences. The present invention thus relates to universal primers which reduce mispriming during cDNA library construction, thereby increasing the proportion of cDNA clones having been primed from the bona fide 3' poly A tail. The present invention further relates to the use of the discriminating oligonucleotides of the present invention in other methods such as mRNA purification, PCR-based detection methods and sequencing.

Poly (A) Tail Internal A-rich Stretch - Uaaaaaaacaaaaagaaaaaaaaa ummunumunu umuminum  $T_{m}$  stable at 37°C -anneals. Tm stable at 37°C -anneals.

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 $Z = 1-(2'-Deoxy-\beta-D-Ribofuranosyl)$ 3-Nitropyrrole

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Oligo d(T)-Z Primer: 5'd(T), -Z-d(T), -Z-d(T), 5'

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Poly (A) Tail Internal A-rich Stretch · Llaaaaaaaacaaaaaaaaaaaaa ttttžtttttttttztttttt uminitali  $T_m$  unstable at 37C - does not anneal.  $T_m$  stable at 37C -anneals.

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